Vol. IV. *January, 1903.* *No. 2.*

BIOLOGICAL BULLETIN.

THE HABITS OF CALIFORNIA TERMITES.

HAROLD HEATH.

Situation of Nest. — Of the six species of termites inhabiting the western part of the United States, three, *Termopsis angusticollis* Walk., *Calotermes castaneus* Burmeist. and *Termes lucifugus* Rossi, occur within one hundred miles of San Francisco. The first named is by far the largest and is seemingly the most abundant. At various points, for example the pine woods not far distant from the Hopkins Seaside Laboratory at Pacific Grove, colonies may be found in almost every stump and decaying log and even dead branches on otherwise healthy trees are frequently infested. As in the case of several other termites their excavations follow more or less closely the grain of the wood and are confined to its deeper portions, leaving an outer protective hull, perforated at various points by tunnels leading from the interior to extensive spaces beneath the bark where the eggs and young are housed during the warmer hours of the day. No galleries lead from the nest into the earth nor into adjacent regions as is the case with *Termes* and if for any reason a portion of a log containing the royal pair be separated even by a few inches from the remainder no intercommunication ever takes place and the latter in due time produces substitute royal forms which maintain the community.

Calotermes castaneus, scarcely more than half as long as the above-described species, appears to be comparatively rare in the central portions of the state. I have taken but two nests, one at Pacific Grove and another on the Stanford University campus, the first in a pine log also inhabited by *Termopsis* though the tunnels of the two did not communicate, the second in a eucalyptus stump. Judging from these two colonies their habits are

essentially the same as those of *Termopsis* though their movements are executed with greater deliberation.

Termes lucifugus, possibly introduced from Europe, has already been the subject of several papers, notably those of Lespes[1] and Grassi and Sandias,[2] and I have only a few observations to add. In California this species is almost as abundant as *Termopsis* and inhabits indifferently several species of oak, conifer and eucalyptus and is sometimes resident in orchard trees and vineyards. Occasionally it attacks the timbers of houses where the moisture is sufficient though its ravages are not serious. During the dry season from June to October, they frequently abandon their haunts above ground and, by means of subterranean tunnels, depart into more favorable regions. On several occasions I have seen them three or four feet beneath the surface and I am told that in some of the more arid sections of the state they sink their shafts to twice this depth. Under such circumstances the burrow does not follow roots as Grassi considers is the case with the European form, but plunges directly into the soil. This and other peculiarities connected with the burrowing habits of this species may be readily observed if a number of individuals are placed in a tall glass jar filled with earth and chips of wood. Almost immediately they commence to tunnel, removing bits of sand and earth to the surface or storing them in cracks with the rapidity of the true ants. Within a week the whole mass of soil is penetrated by a perfect labyrinth of tunnels, which in many places where the walls are in danger of falling in are lined with excrement. In old burrows the walls throughout possess this lining.

In addition to those galleries which pass downward into regions of sufficient moisture there are others that traverse the earth more or less parallel with its surface and sometimes, in the form of tunnels built of particles of wood, come above ground, where they are concealed under sticks and leaves. In one colony inhabiting a large root of the yellow lupine (*Lupinus arboreus*) in a patch of

[1] "Recherches sur l'organisation et les moeurs du Termite lucifuge," *Ann. Sci. Nat.* (4), V. (1856), pp. 227–282, pls. V.–VII.

[2] "Costituzione e Sviluppo della Società dei Termitidi," *Alti dell' Aaccademia gioenia di Scienze Naturali in Catania*, Ser. 4, Vols. VI. and VII. (1893–4). Translation by W. F. Blandford in *Quart. Jour. Mic. Sci.*, Vols. XXXIX. and XL. (1897–8).

loamy soil in a rocky, mountainous district not far from San Jose at least a dozen such tubes existed. One of these, occasionally rising to the surface, led to another root nearly twenty feet away. Another extended in an opposite direction a little over thirteen feet, while some of the other tunnels traversed shorter distances. From these outlying posts other galleries extended and, so far as it was possible to judge, all the roots in that area, nearly an acre in extent, were inhabited by one extensive colony.

Number of Individuals in the Colony. — In such cases and even more where the areas are less circumscribed it becomes practically impossible to determine the limits of a community and the number of members composing it. From this colony just described I took 3,761 individuals — a number probably less than half. On another occasion I took over 1,800 winged forms as they were swarming from a hole almost in the middle of one of the much-travelled streets of Palo Alto. If it be true that the sexes of this species swarm at different times, as Grassi maintains, it becomes evident, in connection with the fact that the winged individuals compose considerably less than half of the colony, that the latter consists, in some cases at least, of over 6,000 inhabitants.

With *Termopsis* the number of individuals is less than in the above described cases. Where the colony is accompanied by the primary royal pair and has been in existence for from one to three and perhaps four or five years its members number from 50 to 1,000. After the death of the primary pair and several substitute royal forms have been developed the egg-laying process is relatively much more rapid and results in the formation of large communities. One such, inhabiting a huge pine log, numbered 3,221 and judging from the thousands of eggs deposited throughout the burrows this number must soon have been greatly exceeded. Under average conditions, however, 2,000 is probably about the usual number.

The nests of *Calotermes* were both small, in one case aggregating about 200 individuals, in the other nearly twice this number. These contained neither eggs nor young and were located in almost completely decayed wood and hence may not be perfectly typical.

Swarming and Founding of Colony. — As is well known, the usual termite colony consists of a royal pair, numerous soldiers,

workers (absent in *Calotermes*) and perfect insects together with larvæ in all stages of growth. The peculiarities of these castes in the California species will be discussed in connection with their breeding habits and the establishment of new colonies.

In the case of *Termopsis* the nymphs (larvæ of perfect insects with plainly visible wing buds) that have developed during the year, undergo their last molt in from ten to twenty minutes, sometimes in the late summer or early autumn, unfold their wings during the next two hours and within a fortnight have changed from relatively helpless individuals to the active, dark-hued insects ready for their extended nuptial flight. This takes place just at dusk and is preceded by a period of great restlessness. From openings which have been made for them by the workers they issue rapidly by twos and threes and, with scarcely any preliminary movements, rise into the air. Some soon fall to the ground but in an open country others may continue their flight fully a mile. At this time they are occasionally beset by numerous woodpeckers, bluejays, tohees, wrens and sparrows who, with much wrangling and chattering, banquet themselves far beyond their usual bedtime. When darkness has compelled them to desist bats continue the pursuit for another hour. So intent are these creatures upon the chase that it is not a difficult feat to strike them down, and in a woodpecker so secured twenty-six termites were found while a bat had captured thirty-three.

Despite these ravages a large number of insects escape and may be seen flying from one tree to another in search of a suitable spot for the establishment of a colony. Several times I have seen them dashing at door knobs and nail holes in houses and against discolored spots on trees and logs in search of a place where decay has begun. Succeeding in this they frequently shed their wings, though with some this act is accomplished almost at the moment of alighting, while with others it may be postponed until after their excavations have reached a considerable size. Curving the abdomen until it rests across the wings of one side they move backward and usually sidewise, thus bringing the tip of the wings against some construction and causing them to buckle and break off along the line of weakness at their base. The wings are never gnawed off.

It appears that in most cases a female selects the site for the

nest and during this process apparently pays very little attention to the one or more males that move about near her. On her falls most of the work of excavation, at least during the first few days, though occasionally when she comes to the outside after a prolonged period of work one of her companions may excavate for a time. In some cases one male appears to be more attentive than the others which often go off on short trips to investigate the cracks and crannies in the neighborhood and may ultimately disappear altogether. In other cases they are driven off by one male and again it sometimes happens that when the burrow becomes a sufficient size to accommodate two, a male takes up a position with the queen and prevents, by fierce lunges, the entry of another. In no case apparently does the female exercise a choice in this first stage of colonization.

Some males are seemingly of a milder disposition than usual, for occasionally one finds two of them in company with one female and, judging by the size of the colony, they have lived together in harmony for more than a year. At other times two females may be associated with one male; and in a few cases I have found two, three and even six pairs living in company. This happens more frequently in captivity, but in any case it apparently disturbs the reproductive process, for the number of accompanying offspring is unusually small. Where royal forms are introduced from other nests a fight almost invariably ensues, and is terminated only with the death of one of the contestants.

It has often been noticed that the antennæ of royal pairs are invariably mutilated. They are, however, intact during the nuptial flight and for three or four days after the entrance into their cell. On several occasions about this time I have seen one individual repeatedly pass one of its antennæ through its mandibles at intervals of perhaps half a minute. Each time it would apparently bite off a small portion and instantly start backward about half its length after the operation. It would then assume a stiff statuesque position with its head rotated to one side or walk with wobbling uncoördinated gait for a little distance before repeating the process. At other times one individual while cleaning the other would gnaw off the tip of the latter's antennæ upon which these same movements would result. The one so mutilated would soon perform the same operation upon the other and so

on in alternation, the final result being the reduction within a few hours of one, often both antennæ, to more or less of a stump-like condition. This operation does not prevent the royal pair from making their way about nor from finding their way back to their cell if removed from it for a distance of several inches. In fact the mutilation in no visible way affects their existence, and it is difficult to imagine of what service it is.

Upwards of a week later than this — that is almost a fortnight after swarming — I have on several occasions seen the royal pair of *Termopsis* in coitu. With their bodies closely appressed end to end in a straight line they remain from one to ten minutes in contact, after which they separate without any external indications that the process had taken place. Several times from a few days to many months later than this I have witnessed the same process in various colonies. With one pair kept in captivity this occurred at least a dozen times during eleven months and probably happened more frequently, though in a fairly high temperature (from 20–24° C.), such as is developed by placing them in close proximity to a lamp, this appears to be a much more frequent occurrence than in their normal cool moist habitat.

The swarming of *Calotermes* takes place in January and is quite similar to that of *Termopsis* as is the pairing process. Owing to the fact that I have had but one colony at a time new colonies were not established, but the males and females of the same community not only paired as though more distantly related, but mated and laid eggs. These were non-fertile, however, and soon grew white and disintegrated. *Calotermes* is rather difficult to keep in captivity, but some of these royal pairs lived for nearly five months and assumed almost exactly the size and appearance of the true royal progenitors of the original colony (Fig. 1) which were present in each of the two nests.

Termes lucifugus swarms at different times between the months of October and April. I have watched it more than a score of times and can confirm Grassi's observations in all essential particulars. It usually takes place about 11 A. M. and lasts nearly two hours. During its occurrence a few birds make their appearance and they, together with lizards (*Sceloporus*) and *Formica rufa*, reduce the size of the swarm somewhat though not seriously.

After their short flight the wings are almost immediately shed and the greater number of individuals pair, the male following immediately behind the female. According to Grassi (Blanford's translation, *t. c.*), "the one in front attempts to run away from the other, which pursues it and palpitates the extremity of its abdomen, and sometimes the sides as well. . . . I believe that the meaning of these supposed amorous displays is entirely different from that usually assigned to them, and that the pursuer wishes to solicit the dejecta from the one pursued."

FIG. 1.

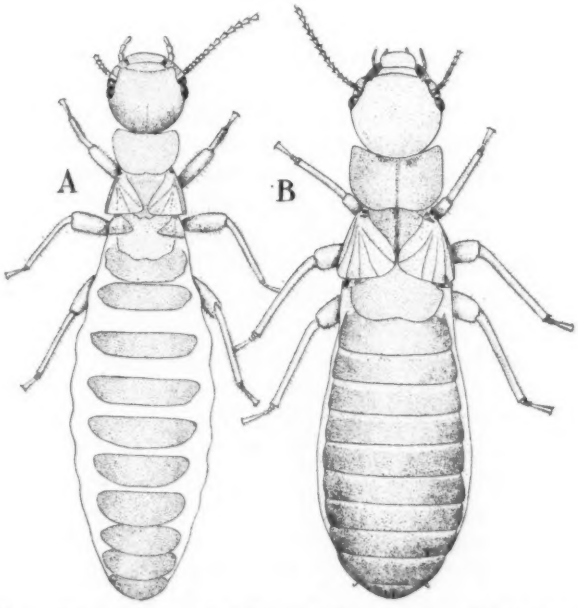

FIG. 1. Primary queens of (*A*) *Termes lucifugus*, eight months old, × 14; (*B*) *Calotermes castaneus*, probably not less than four years old, × 9.

This may be the correct explanation, but there are some facts that throw much doubt upon it. In the first place no food is taken by the perfect insect from the time of its last molt until the work of excavation has commenced and dissections show the alimentary canal to be almost empty. Secondly when one termite

solicits dejecta from another it scarcely ever pursues it for more than a centimeter unless there are signs that the scybalum is forthcoming, and even then the pursuit is only for a short distance. But here in the case of *Termes* one perfect insect often pursues the other for many meters, and I have noticed in the case of several which fell in some large cement basins that they kept it up for an entire afternoon and evening. Furthermore, if the individual behind becomes separated from the one in front the latter almost immediately stops and stands transfixed with the abdomen curved upward until it once more feels the palpitation of the antennæ of the one behind. The whole process suggests that some secretion located at the posterior extremity of the body attracts the one behind until the other finds a suitable spot for a nest.

Within a short time after mating the female burrows into the soil where this is soft; otherwise she traverses some crevice and disappears from sight followed by the male. In Italy, according to Grassi, their subsequent careers are without result. Nevertheless, this investigator was able to obtain the commencement of a colony and Perris[1] notes that in the Landes royal pairs accompanied by eggs are of frequent occurrence under chips. In California this species is very difficult to keep, owing to its inability to withstand even slight changes in the amount of moisture in the nest. It thrives best in large jars filled with chips and earth, but even in this habitat it rarely lives over three or five months. Royal pairs, when placed in these surroundings, after swarming, disappear almost immediately and the following day may be seen next to the bottom of the jar a foot or so from the surface, excavating hollows in the wood and neighboring soil. Five weeks later I have found small clumps of eggs which in one colony hatched, but the young as well as the parents soon died.

Under natural conditions it is almost impossible to find these royal pairs, owing to their habit of burrowing in the soil, but I have secured four colonies after upwards of a year of watching. Three of these occupied excavations in some old fence posts almost completely buried in an unused gravel pit near San Jose. Placed in essentially the same conditions one and a half to two

[1] Ann. Soc. ent. France (5), VI., 1876, p. 201.

and a half feet beneath the surface they contained seven, nine and thirteen eggs and six, three and eight young respectively. The other colony was placed under and in a plank buried about a foot beneath the soil near Stanford University and contained the true royal pair, seven eggs, one soldier and twenty-seven workers and young of different sizes. From these few examples I am strongly of the opinion that the establishment of new colonies by means of true royal pairs is not only a normal process in California but very probably one of comparatively frequent occurrence.

As previously noted the true royal forms of all three species here considered, abstain from any form of nourishment from the time of their last molt until the process of excavation has begun, and no special supply of food is carried from the paternal roof in order to enable them to exist until the time arrives when they may be cared for by their offspring. On the other hand everything at this time is apparently sacrificed to lightness of body, resulting in a wider dispersal with less exhaustion than would result with forms loaded with fully developed reproductive organs or food either in or out of the body.

A very short time after excavation has commenced the abdomen of both male and female increases rapidly in size. That of the latter is slightly larger and more globular, but in both the enlargement is due to nutriment taken into the alimentary canal and to the developing reproductive elements. Within a month both have attained to nearly their maximum size which, as the figures show, is far short of that of some of the tropical forms. At no time do they require to be fed, and their size does not prevent their active locomotion from place to place.

Rate of Increase, etc. — As previously mentioned eggs are deposited in the newly made nests of *Termes lucifugus* within five weeks after pairing, but owing to the fact that these and the young are shifted about in galleries often away from the light, the observations I have made concerning the rate of egg-laying and the time required for hatching are very incomplete. This is also the case with *Calotermes*. *Termopsis*, on the other hand, is much more hardy and lends itself readily to observation and this, connected with their abundance, has placed me in possession of considerable data. They mate readily in captivity, and upwards of

500 true royal pairs now in my possession are in excellent condition after more than a year of captivity. They are also very abundant in recently killed trees, and it is an easy matter to use these colonies for checking up results. Stripping off the bark of such trees from three to nine months after the swarming period I have found scores, in fact hundreds, of small colonies accompanied by the royal pair.

Up to the present time no record has been made of the occurrence of a true royal pair within the United States. Messrs. Banks and Chittenden have kindly called my attention to a brief note by Hagen[1] to the effect that the late Mr. H. G. Hubbard found the queens of *Termes flavipes* in Florida. In this note Hagen remarks that the "females are undeveloped, being wingless, but sexually mature," which shows that they were substitute and not true royal forms. The royalty of such small species as *Termes* and *Calotermes* might easily escape detection, but as *Termopsis* is relatively huge and also abundant and widely distributed throughout the Pacific slope it is remarkable that they have not hitherto been discovered.

For *Termopsis* the date of egg-laying and the consequent development of a colony differs widely according to circumstances. When the habitat is unusually moist a royal pair may remain together for a year without producing offspring, and, on the other hand, under favorable conditions upwards of 75 young may be developed within this period of time. Usually a fortnight elapses from the time of pairing until the first egg is laid; and from one to six are deposited daily until the total number amounts from 15 to 30. Egg-laying now ceases and the parents give their time to enlarging the nest or to caring for the eggs which are objects of careful attention, being kept scrupulously clean and frequently shifted from place to place in the nest.

At the present time my observations are not sufficiently complete to justify an extended account of the rate of growth of the developing young, the number of molts they undergo and the origin of the various castes. With the primary royal pair these phenomena are continually subject to a gradual change. For example, early in the life of the colony a large-headed larva

[1] *American Naturalist*, Vol. 10, 1876, page 62.

arises as a result of what appears to be the third molt, and after three succeeding ecdyses it becomes a perfectly formed soldier. The latter, in long-established communities, is about 18 mm. in length and possesses 22 antennal segments, while the one first developed is but 10 mm. in length and has 18 or 19 segments. Upwards of three months later another immature soldier appears larger than the first and evidently formed as a result of more than three molts. More than three are also apparently required to give it the adult length of 12 mm. and usually 19 or 20 antennal segments. The new arrival, after the mandibles have become sufficiently chitinized, usually asserts its superiority by putting an end to the first. Other soldiers appear upon the scene from time to time, generally a little larger and occasionally with more antennal joints. Finally an equilibrium is established in this respect, but not until the colony has been established for a year at least.

The first workers also undergo their final molt at an early stage and, as with the soldier, those which appear later assume larger proportions until, finally, at the end of nearly two years, a limit is reached with some of the members which have attained a length of approximately 14 mm.

So far as I have seen nymphs do not occur until after the first year, in other words the first swarm of perfect insects leaves the nest at the end of the second year. They also are relatively small.

The life of all these first inhabitants is comparatively short. The king and queen early draft them into service, but when their place may be taken by other and more powerful individuals they disappear. In some cases they are undoubtedly dispatched, but it is by no means certain that this is always the case. After the colony is fully established soldiers and workers live at least two years and probably longer, though of this I cannot be positive. Regarding the king and queen it is certain that they live together for at least two years in the case of *Termopsis*. In three of the largest two-year-old colonies in my possession there are in the neighborhood of 200 individuals, while in some colonies headed by the royal pair I have found more than 1,000. From such nests many perfect insects have swarmed, and it is reasonable to

believe that they have existed for five years at least. There is not the slightest evidence that the kings do not live as long as the queens. After the death of the royal pair from natural or other causes the members of the orphaned colony (to use Grassi's expressive term) develop a number of substitute royal forms. The individuals chosen for this purpose are usually the larvæ of perfect insects in which the wing buds are barely visible. By some unknown process, possibly by a peculiar method of feeding or some change in their diet, the external larval characters are retained (the larvæ undergoing no subsequent molts), while the reproductive organs are stimulated to active growth culminating in full functional activity. Very shortly, often within a month after the death of the primary pair, these substitute forms may be detected, owing to their faint straw color, which rapidly grows to a very much darker tint during another month.

If only one of the royal pair be destroyed usually only one substitute form is developed, but when both perish from ten to forty substitutes appear, according to the size of the colony. In two nests carefully examined I found six substitute males and eleven females in one, and nine males and thirteen females in the other. As might be expected, these do not mate permanently, and one male may pair with three or four queens during the course of an hour.

The substitute individuals are fed and cleaned almost entirely by the workers, which also assume the care of the young. Accordingly the royal task consists almost altogether of egg-laying and is performed with comparative rapidity. Some of the larger queens (Fig. 2) lay continuously from seven to a dozen eggs in twenty-four hours and where several are associated together the colony rapidly assumes large proportions. In the extensive nest of *Termopsis* mentioned on page 48 there were over thirty substitute individuals which were congregated beneath a small strip of bark where the sun shone brightly, and with them was a clump of eggs, attended by many workers, that I carefully estimated at not less than 8,000.

In colonies where either the king or queen persists the substitute royal individual is invariably, so far as I know, an immature perfect insect, but where both have perished the substitute royalty

may sometimes contain a worker or a nymph or even a soldier capable of laying eggs. Such monstrous forms are not infrequent in large orphaned nests, but never apparently in colonies headed by the true royal pair. One may also find winged soldiers, soldiers with mandibles of varying size, and, as just mentioned, soldiers with wing buds, the straw-color characteristic of substitute forms and with functional reproductive organs. These

FIG. 2.

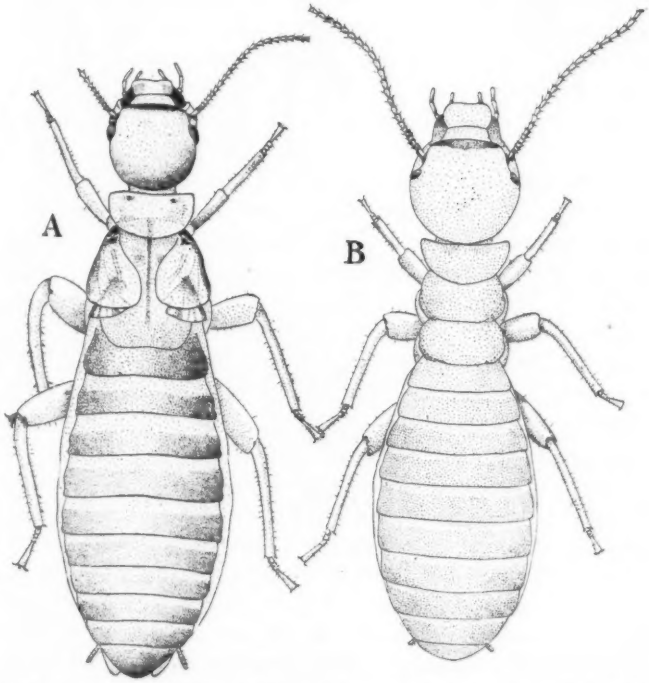

FIG. 2. *A*, primary queen of *Termopsis angusticollis*, at least four years old, × 6; *B*, complemental queen of same species, × 7.

last-named insects are comparatively rare. I have had but three in my possession. All of them laid eggs in captivity and in one case I followed the development for a long period of time, but the young and the nymphs and workers into which they became transformed, appeared in all respects perfectly normal.

Food, etc. — The food of termites consists almost wholly of wood gnawed directly from the wall of the burrow or solicited from some other individual. The primary royal pair appear to live almost altogether on food which they scrape off for themselves, When the young are hatched they are fed for some time on material regurgitated by the parents, but after a molt or two proctodeal food is added and finally constitutes almost the entire food supply procured from another individual.

After having fed a colony on oak or redwood (*Sequoia*) which produces dark-colored dejecta, and then changing the diet to white pine, it is easy to see that the greater part of the excrement, voided in the form of white pellets, never again enters the digestive tract of another individual. Apparently this is chiefly owing to its dryness. Some specimens, probably all at different times, appear to have the power of retaining a certain amount of moisture in the excreta. These are eagerly sought out by their companions which palpitate the abdomen of the one solicited with their antennæ and clasp its tip between their mandibles. If the stimulus be too early applied the one sought after runs away; otherwise it passes out the pellet which is greedily devoured. Occasionally one may feed itself in this way. This proctodeal food supply constitutes almost the sole diet of the substitute royalty, whose dejecta, like that of the soldiers, is never again devoured.

It appears to be a general belief that the soldiers, on account of the huge size of their mandibles, are able only with the greatest difficulty to solicit proctodeal food, and are accordingly in a chronic state of hunger. Sick or disabled individuals are greedily eaten by them, and at various times perfectly healthy forms are dispatched and devoured. Grassi supports this belief by some examples, but at the same time he notes that *Calotermes flavicollis* also solicits food in the usual way. With all the Californian species the soldiers are almost invariably at peace with their fellows. Moribund and possibly supernumerary forms are dispatched, but this is rarely done by the soldiers, who stand guard most of the time at some of the more exposed parts of the nest. At other times they move quietly among the workers and nymphs, and, with heads rotated to one side, solicit food with

very little difficulty. After watching many colonies for more than a year I have yet to see a soldier subsisting on any other kind of food. On one occasion I saw a soldier of *Calotermes* trying industriously to gnaw off part of the abdomen of a disabled nymph and at another time watched one biting a splinter of wood, but these efforts were of short duration and without success.

FIG. 3.

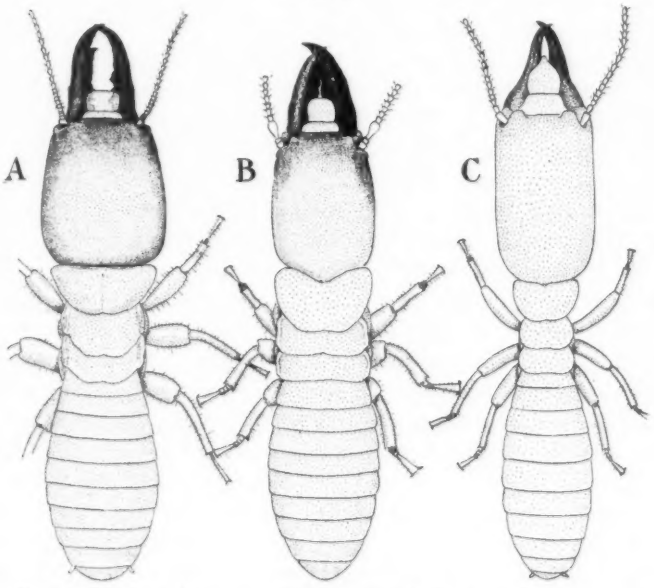

FIG. 3. Termite soldiers. *A, Termopsis angusticollis*, × 5; *B, Calotermes castaneus*, × 9; *C, Termes lucifugus*, × 12.

On several occasions, while transferring colonies from their nests into jars, I have seen the soldiers of all three species rush frantically about and impale one of their fellows on their mandibles, but after quiet had been restored such acts were never repeated and it is safe to conclude that they form no part of the normal life of a colony.

In the presence of sufficient moisture the discarded rejectamenta are often used in the construction of barricades or for filling old beetle burrows; otherwise they are cast outside the nest.

Some species are said to cement the particles together by means of saliva or material regurgitated from the anterior part of the alimentary canal, while others employ the method of *Termopsis* and *Calotermes*. These, carrying a pellet in their mandibles, travel to some spot where building is required, and, feeling about with their antennæ, locate a breach in the wall. Facing about they then deposit a drop of liquid proctodeal material into which the excrement is quickly pressed with many waggings of the head, and immediately rush after another pellet which is likewise stuck fast. By this time the cement has set and the worker or nymph departs into the crowd, where it rests and is groomed by its fellows. In their natural surroundings the amount of building done by these insects is relatively small, but colonies kept in glass cases and in the presence of sufficient moisture, erect low but extensive roofs, elevated on many pillars, that serve to protect them from the light and other injurious agents.

It is now thoroughly well established that the soldier and worker in the termite colony are not the result of the arrested development of the reproductive organs. It has also been shown that they are not restricted to either sex. And it is almost equally certain that their differentiation is not to be traced back to the newly hatched young. The latter, when they first appear, are exactly alike in form and color, though they may exhibit slight differences in size, and the characteristics of the different castes develop at varying times after the first molt. If then neither arrested development, nor sex nor heredity are directly responsible for the production of soldiers and workers, what is the agent immediately concerned? Grassi is of the opinion that it is the food. Owing to its character or amount or both, the royal pair or the colony are able to transform the larvæ into soldiers, workers or perfect insects. Such a belief gains some support from allied phenomena among the social hymenoptera, but at the present time very little definite knowledge exists regarding this subject for the termites. For months I have fed a large number of termite colonies of all ages, with or without royal pairs, on various kinds and amounts of food — proctodeal food dissected from workers or in other cases from royal forms, stomodeal food from the same sources, sawdust to which different nutritious in-

gredients had been added—but in spite of all I cannot feel perfectly sure that I have influenced in any unusual way the growth of a single individual. However my experiments along this line are far from being completed and it appears best to hold the account of this phase of the subject for a future time.

STANFORD UNIVERSITY, CAL.,
October, 1902.

A PRELIMINARY NOTE ON THE OCCURRENCE OF A FILARIA IN THE CROW.

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The discovery of a number of species of filariæ in birds in southern Nigeria in 1900, when studying the hæmamœbæ, as a member of the Liverpool Malaria Expedition to Nigeria, suggested the question whether these parasites were to be found in birds in temperate climates.

Few opportunities offered in the course of my duties, but a few bluejays and crows were secured during the summer of 1901. The bluejays were examined with negative results. Of eleven crows three were found to have embryo filariæ in the blood, all of one species. In two of the three the red corpuscles were infected with *Halteridium*.

The search for the parent forms of the filariæ was not successful, and owing to the migration of the crows, further study had to be deferred until the following summer.

This year I have been able to secure only four crows. One of these fortunately was infected with the same filaria found in 1901. After careful dissection and teasing of the tissues the parent forms were found in the coats of the pulmonary artery. In appearance they resemble the human form *F. Bancrofti*.

The embryos vary greatly in quantity in the blood stream of the different birds. In the fresh preparation their movements are rapid, and they progress readily in either direction. By ringing with vaseline they will keep active for several days. The average length of a number measured in the fresh preparation was 187.96 microns. In permanent cover-glass smears, dried, fixed in alcohol, and then stained, the length varies somewhat, averaging 173.1 microns.

What appears to be the head end is blunt or abruptly rounded, the anterior two thirds being of uniform diameter. The posterior

third tapers gradually until it reaches about one half the width of the anterior end, when it is abruptly rounded. In the fresh condition no sheath could be made out nor does it show in preparations stained with fuchsin, but with hæmatein a sheath is well defined extending however only slightly beyond either the head or tail.

In stained preparations .05 of the anterior end is clear; at .37 from anterior end and extending to .4 there is in most specimens a paler spot in which there are few nuclei; at .6 from the head end, and extending to .7 there is a clear spot in all the specimens in which no nuclei are seen, and little or no stain is taken. This spot and its fixed position is characteristic of this embryo, and clearly distinguishes it from any filaria which has been previously described. At this spot in the stained specimen the diameter is 5.8 microns, as compared with 4.7 microns at the blunt end, and 3.4 microns at the tapered end.

I hope very shortly to give a detailed description of this new filaria, and the embryos found in the circulating blood.

GRAVENHURST, ONT.,
October 16, 1902.

THE ORIGIN AND DEVELOPMENT OF THE GASTRIC GLANDS OF DESMOGNATHUS, AMBLYSTOMA AND PIG.

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INTRODUCTION.

A precursory study of the glands of the pig's stomach successfully demonstrated at the very beginning of this investigation the difficulties attendant upon a true understanding of the real origin of the stomach glands from the study of such a complex form. I am glad, here, to acknowledge my indebtedness to Dr. B. F. Kingsbury, who suggested the form *Amblystoma*, and to Mr. C. H. Boxmeyer, who suggested, and assisted me in procuring the material for the study of *Desmognathus*. It is to the study of this latter form I ascribe any success I may have had in this investigation.

Many investigators have been interested in the origin and function of the different cells, as parietal and central, in the glands of the stomach, but few have studied the development of the glands for itself alone. Thus only can one account for the lack of accurate information on this subject. The present paper goes, perhaps, to the opposite extreme, in being concerned solely with the origin of the glands from the earliest recognizable stages, until it can be clearly demonstrated that true glands are formed. In *Desmognathus* this has been comparatively simple, in *Ambly-*

stoma, less so, on account of the great number of yolk spherules obscuring all details; while in the pig the complex structure of the glands has rendered this especially difficult.

Technique.

As both *Amblystoma* and *Desmognathus* abound in Ithaca a complete series of each was obtained. The material was fixed every other day and accurate measurements taken, so that a full series, differing less than ½ mm. in total length between each, was secured. Fixation was accomplished in various ways: Kleinenberg's picro-sulphuric (distilled water, 100 c.c.; sulphuric acid, 2 c.c.; picric acid to saturation, 1 part; distilled water, 3 parts), Flemming's chrome-acetic (water, 19 parts; 1 per cent. osmic acid, 16 parts; glacial acetic acid, 2 parts; 10 per cent. chromic acid, 3 parts), picro-nitro-sublimate (sat. aq. sol. of picric acid, 500 c.c.; water, 300 c.c.; nitric acid, 24 c.c.; mercuric chlorid to saturation), and Perenyi's fluid (10 per cent. aq. sol. of nitric acid, 4 parts; 95 per cent. alcohol, 3 parts; ½ per cent. aq. sol. of chromic acid, 3 parts) all gave fairly good results. But Carnoy's, modified (glacial acetic acid, 30 c.c.; absolute alcohol, 30 c.c.; chloroform, 30 c.c.; nitric acid, 3 c.c.; mercuric chlorid to saturation), fixing ten to sixty minutes, washing in 67 per cent. alcohol, then in 82 per cent. alcohol and iodin, and Gilson's, modified (glacial acetic acid, 5 c.c.; nitric acid, 5 or 10 c.c.; 95 per cent. alcohol, 100 c.c.; sat. aq. mercuric chlorid, 400 c.c.), fixing three fourths to twelve hours and washing as in Carnoy's, gave the most satisfactory results.

Many different stains were used, as methylene blue and eosin, eosin and orange G, carmine-picro-nigrosin, thionin, safranin, iron hematoxylin, but for staining sections on the slide chloral hematoxylin with eosin as a counter stain gave the best results. It was also found very advantageous to stain material fixed in Gilson's or Carnoy's in toto. Eosin, restaining with hematoxylin and eosin, or borax-carmine (Grenacher), restaining with hematoxylin, proved satisfactory. Staining in toto with Delafield's hematoxylin and counter-staining in toto with a saturated alcoholic solution of eosin yielded excellent results, as there is very little danger here of overstaining. (Small pieces were stained

24–36 hrs. in Delafield's hematoxylin, Del. hem., 1 part, + distilled water, 4 parts, then washed in distilled water 10–12 hrs., or until the water is no longer colored by the hematoxylin. They were then stained in toto with eosin, sat. sol. in 95 per cent. alcohol, washing out with absolute alcohol.)

For the earlier stages, on account of the great amount of yolk present, double imbedding with collodion and paraffin was employed. Thinner sections could be obtained in this way, with less loss of yolk material. In thickness the sections varied from two to ten micra.

The material used in the study of the stomach of the pig was placed at my disposal by the Department of Histology and Embryology. It is a great pleasure to express my appreciation to the members of this Department for the interest shown during the investigation.

This material was fixed in five per cent. formalin, mercuric chlorid, alcohol, picric alcohol and Zenker's fluid. In spite of the tendency of the epithelium to shrink away from the mucosa the formalin material proved the best for study. Staining in toto with alcoholic eosin, restaining with hematoxylin and eosin, or staining in toto with Delafield's hematoxylin and alcoholic eosin was employed with good success. Other stains as mentioned above for *Desmognathus* and *Amblystoma* were also used.

All figures are drawn with an Abbé camera lucida. Unless otherwise stated drawings are made with Leitz lenses and the ordinary tube length. Objective 1/16, oil immersion.

Origin of the Gastric Glands.

Desmognathus.

Notwithstanding the general tendency of to-day to explain the development of the so-called higher forms by comparison with the simpler or lower, nothing has been done, as yet, along this line, for the gastric glands. The embryologies are singularly free from references as to the origin of glands, save in the higher forms, and even here there is a dearth of helpful literature.

To review, briefly, the facts, as known in the Batrachian forms so far investigated, will aid to simplify the presentation of this

subject. The œsophageal glands of *Urodela* have received more or less consideration as the question of the homology of these glands, with one another, and with those of higher forms has for a long time aroused much interest. But, leaving this interesting problem entirely out of the discussion, there are described in the stomach of some of the *Urodela* two kinds of glands, the anterior and posterior oxyntic glands of Langley ('81). Bensley ('00), for *Amblystoma*, and Carlier ('98), for *Triton*, have given excellent descriptions of these glands. In the adult form the

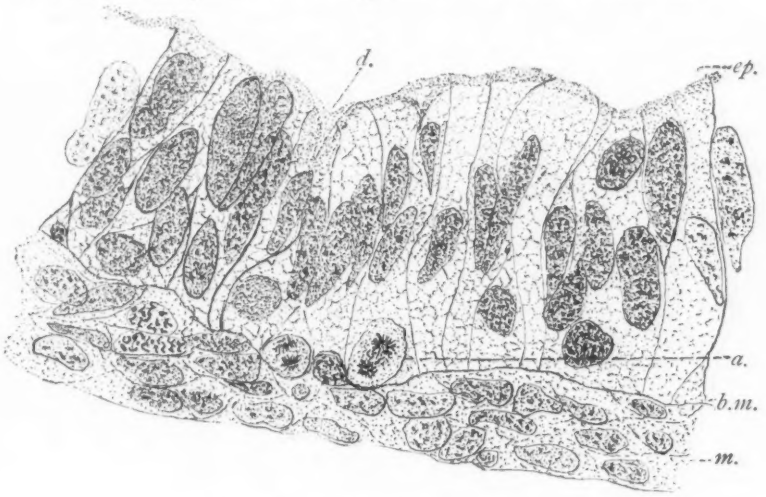


FIG. 1. Transection through the fundus of the stomach of a larval *Desmognathus*, 11.5 mm. Fixed in Gilson's, modified. *a.*, gland anlage, "ovoid cell," just about to divide; *b.m.*, basement membrane; *d.*, insinking at the surface of the epithelium to form the future excretory duct; *ep.*, epithelial lining of the stomach, showing one of the cells in process of division; *m.*, mesodermic tissue forming the muscular layers about the stomach. This also shows mitoses. Obj. $\frac{1}{8}$, Oc. 1.

glands consist of a duct, composed of cells resembling the surface epithelium, a neck, composed of clear mucous cells, and a body, made up of granular cells containing large nuclei. The glands of the cardiac, fundic and pyloric regions vary in complexity but all resemble, to a greater or less extent, the type form just described. These anterior oxyntic, or cardiac glands, may or may not be transition forms between the true gastric glands and

the large œsophageal glands. But, whatever they may be as to homology, all of the glands of the stomach are alike in the formation of their early stages.

The early stages in the formation of the gastric glands in *Desmognathus* are easy to discern, as in this form there is comparatively little yolk present to obscure the cell outlines. The first traces of the future glands are found in a larva of 11 mm. total length. The cells are at first apparently many layers thick, but on careful inspection it may be seen that they are arranged in an orderly manner like simple columnar epithelium as shown in Figs. 1 and 2. The nuclei, thus arranged at different levels,

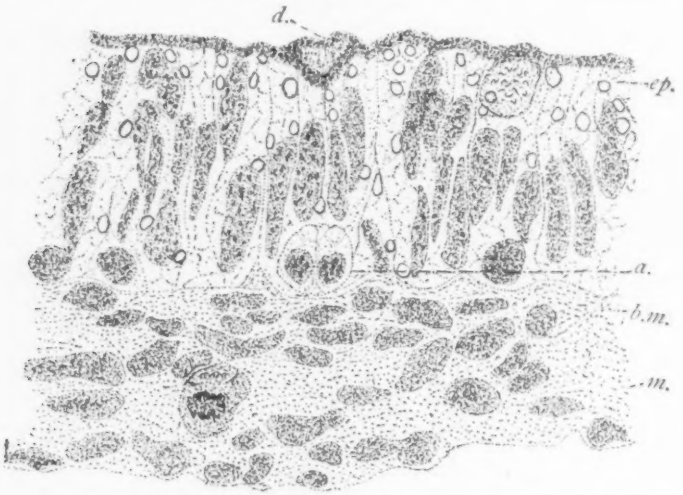


FIG. 2. Cross-section through the cardiac region of the stomach of a larva 11.5 mm. long. Gilson's. Letters as in Fig. 1. This shows a round cell dividing in two. Obj. $\frac{1}{12}$, Oc. 2.

give to the epithelium a stratified appearance, which is somewhat misleading at first sight. In the same figures insinkings or slight indentations on the free surface correspond to the cells whose nuclei lie deep in from the surface (Figs. 1 and 2, *d*). At the base of the surface epithelium, resting almost upon the basement membrane, are large round cells, with round or oval nuclei (Figs. 1, 2, *a*). These cells and nuclei are larger, clearer, and more

finely granular, resembling the "ovoid" cells described by Sewall ('82) in the sheep, and the "ersatzzellen" described by Toldt ('80) in the cat. In sections stained with hematoxylin and eosin the nuclei of these cells take a deep blue, and the cells a deep red color. This enables one to trace the changes in these cells in *Desmognathus* with comparative ease. Even in larvæ less than 11 mm. in length these round cells may be observed. They

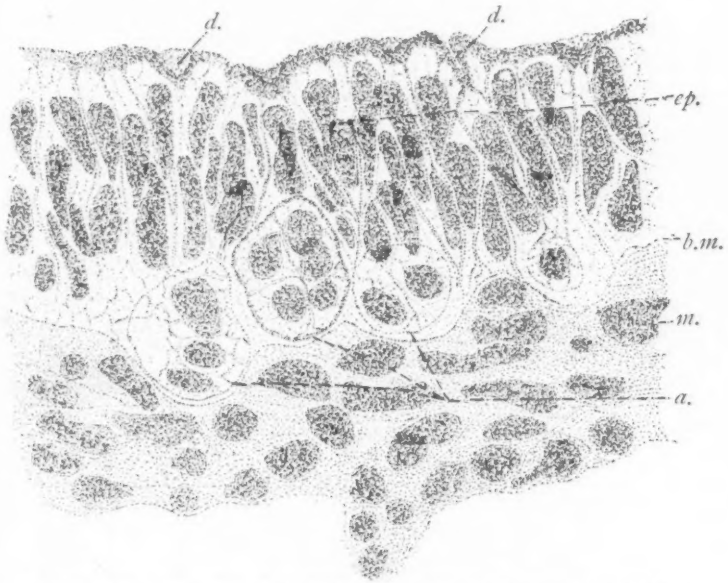
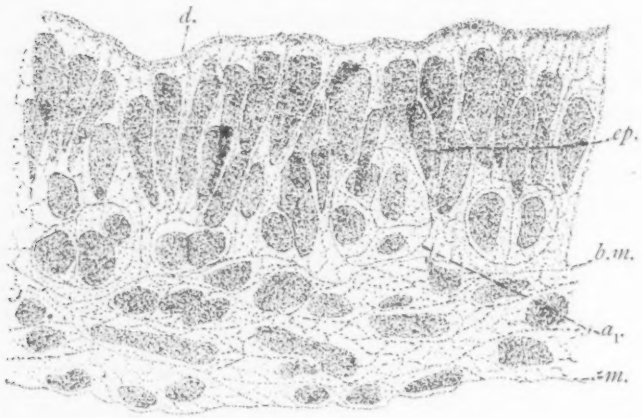


FIG. 3. Cross-section through the fundus region, where the pancreas is attached to the stomach; *a.*, gland fundaments in various stages of development, forming groups of several cells. By their growth they are displacing laterally the cells of the surface epithelium, *ep.*, making the insinkings more marked, *d.* Other letters as in Fig. 1. Obj. $\frac{1}{16}$, Oc. 2.

appear with the differentiation of the stomach from the general entoderm. These cells then divide, as shown in Figs. 1, 2 and 3, forming groups of two, three and four cells. In Fig. 1 are two of these cells in a state of division. All of the cells of the epithelial and subepithelial layers appear equally capable of multiplication but the gland anlages at this stages show marked

mitoses. Thus by repeated division these small rounded masses of cells increase in size. It does not appear probable that these



FIGS. 4–7. The following four sections form a series representing one gland throughout its whole extent. Transections (10 μ) through fundus of a larva of 13 mm. Fixed in Gilson's. Letters as in Fig. 1. Obj. $\frac{1}{12}$, Oc. 1.

FIG. 4. a_1, end view of gland, showing some of the epithelial cells of the surface projecting around the end of the gland.

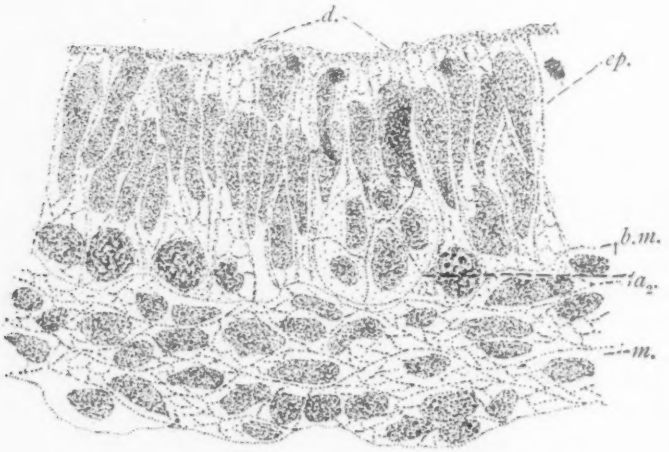


FIG. 5. a_2, transection through the center of the gland.

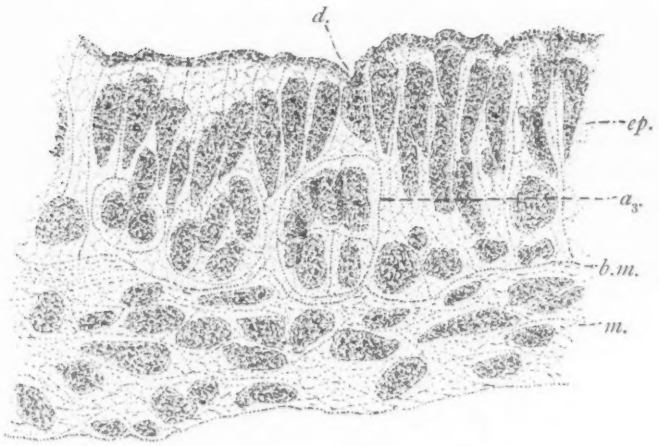


FIG. 6. a_3, same as Fig. 5. No lumen shows as yet.

small round cells at the base of the epithelium coalesce to form a gland, as no trace of such aggregation could be found, but that each cell gives rise to an individual gland. While this process is

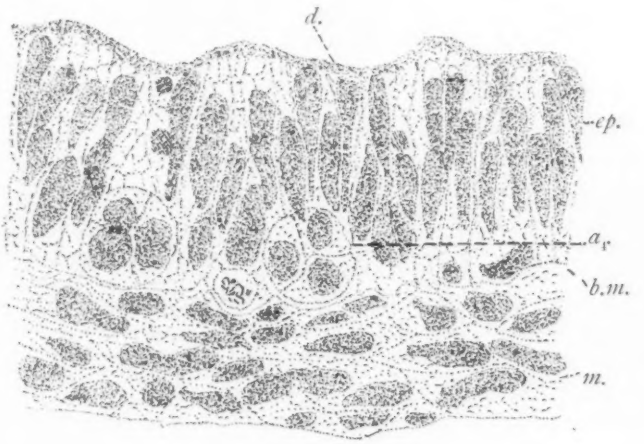


FIG. 7. a_4, same as Fig. 4.

going on the insinkings at the surface (Figs. 3, 10, *d*) continue to deepen, forming, in *Desmognathus*, narrow tubes. The gland fundament, by rapidly increasing in size, is forcing its way, not down into the mesodermic layer, though there is a slight burrowing in this direction (Figs. 1, 3, *a*), but toward the lumen of the stomach. The epithelial cells of the surface are being pushed out of their original position, and arranged around the gland fundament as a central axis (Figs. 3, 4, *ep*). As the free surfaces of

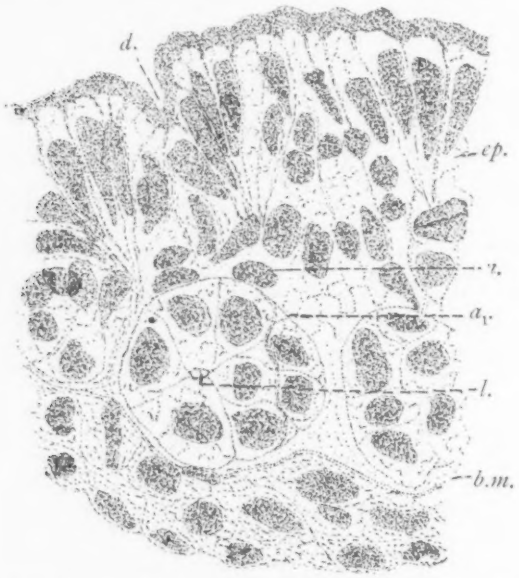


FIG. 8–11 represent a series of sections through a gland of the pyloric region of the stomach of a 16 mm. larva. Fixed in Gilson's. Letters as in Fig. 1. Obj. $\frac{1}{8}$, Oc. 1.

FIG. 8. a_1, transection through a gland fundament showing the lumen; *d.*, the insinking at the surface is more marked than in earlier stages; *n.*, cells which later take part in forming the neck.

the cells are thus withdrawn from facing the cavity of the stomach to form the lumen of the gland, or, speaking more accurately, to form the excretory duct, the epithelial cells adjoining the gland proper, or fundus, become rounder and appear in a more active condition than the rest of the epithelium. This is shown in Figs.

4, 5, 6 and 7 of a larval *Desmognathus* 13 mm. long. The gland fundament here extends laterally throughout at least four sections (40 micra), or, perhaps, six as it is extremely difficult to trace out all the individual cells of such a series. As yet there is no lumen apparent in the gland (Figs. 1–7). A section through the center of the gland is shown in Fig. 6. This shows the insinking (*d*) at the surface, and the arrangement of the nuclei of the surface epithelium around the gland as an axis (*a*). Fig. 4,

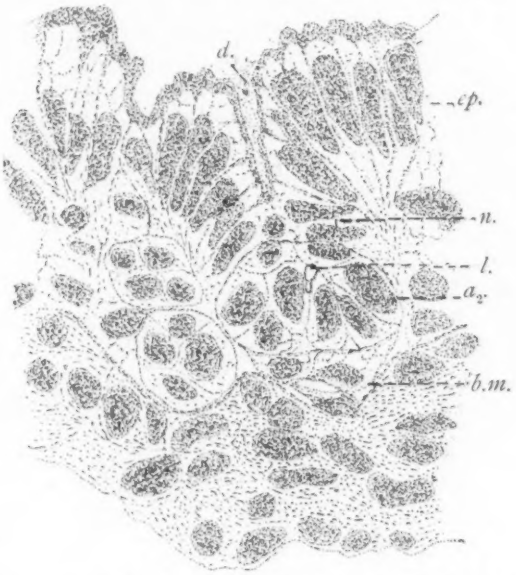


FIG. 9. Same as Fig. 8. a_2, the cells of the gland fundament are arranging themselves about the gland lumen in a way to indicate the approaching union of the duct and fundus at *n*. (the neck cells); *ep.*, the epithelial cells show the lateral displacement, which gives rise to the duct, to a marked extent.

a section nearer the edge of the gland, shows the arrangement of the nuclei of the cells around one end of the gland (*a*). This same series (Figs. 4–7) also shows another interesting fact, which indeed holds true for all the animals studied. While the stomach is being differentiated from the entoderm all the above processes are taking place at the same time, so that in the same series are found gland fundaments in different stages of development. So,

as in Figs. 4, 9, 12, the more advanced and earlier stages are often seen in the same section. In no stage, however, were the two extremes, *i. e.*, a completed gland and a beginning one, found.

In a larva of 16 mm. the stomach is entirely formed, but as yet no glands open on the surface. Figs. 8–11 show glands further developed than those just described. By the increase in size of the gland and the multiplication and rearrangement of the cells

FIG. 10. Same as Fig. 8.

lining the stomach, the excretory duct is being formed (Fig. 9, *d*). Before the connection between the fundus of the gland and the excretory duct is established, a lumen is formed in the gland fundament (Figs. 8, 9, *l*). This appears to be caused by a rearrangement of the cells and not by a cell disintegration. The connection between the duct and fundus, forming the neck of the gland, is also brought about by a rearrangement of the cells and not by a cell disintegration. In Figs. 12–17 some of the glands

open to the cavity of the stomach. The neck cells, connecting the excretory duct and fundus, appear in a strongly active condition (Fig. 15, *n*). This maturing of the glan occurs just at the time of hatching, so that when the *Desmognathus* is hatched some of the glands are completely developed (Fig. 13, *n*) while others are still incomplete (Fig. 13, a_2). Several days after hatching all the glands open to the surface of the stomach. When

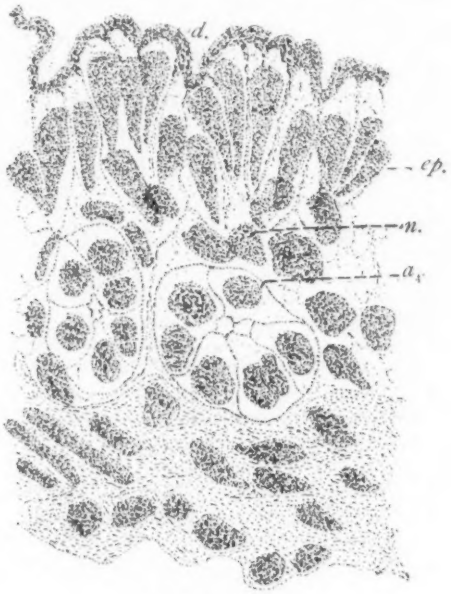
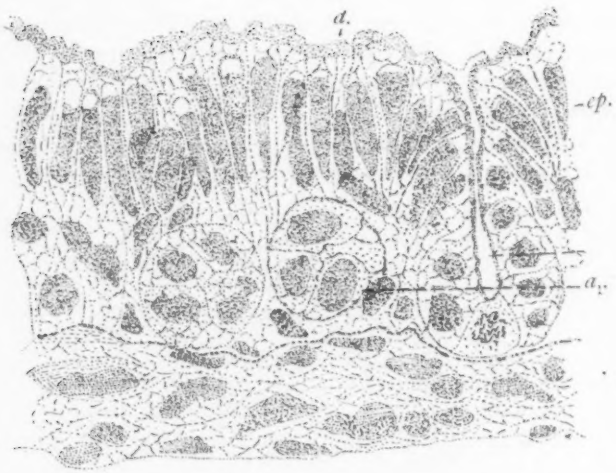


FIG. 11. Same as Fig. 8.

this has taken place no more glands are formed in the way just described, but further increase is by a subdivision of the fundus of the gland, in the same manner as later described for the pig.

Amblystoma.

In the *Amblystoma* the gland anlages are not readily demonstrated as the cells of the epithelium lining the stomach are completely filled with yolk. Staining the sections on the slide with hematoxyln, eosin and picro-fuchsin aided materially in over-



FIGS. 12–17 represent a series through a gland, showing the union of the duct and the fundus. This is taken from the central region of the stomach of a larva *Desmognathus* at the time of hatching. Glands in all stages of development, from the one-celled anlage up to a completely formed gland, are shown thoughout the sections. Fixed in Gilson's. Obj. $\frac{1}{12}$, Oc. 1.

FIG. 12. a_1, transection showing merely the outside cells of the gland.

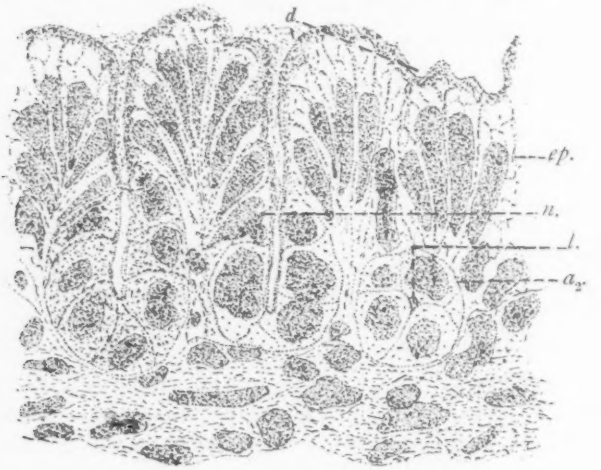


FIG. 13. a_2, transection showing lumen of the gland, *l*.

coming the difficulties caused by the large amount of yolk. The nuclei took a deep blue color, the yolk spherules became a transparent orange yellow, while the round cells, so characteristic of this stage in the *Desmognathus*, stained a deep red. In a larva 11 mm. total length, although the caudal portion of the stomach is yet undifferentiated, these large, granular, round cells are found at the base of the epithelium (Fig. 18, *a*). Fig. 20, *a*, shows one already divided, while in Fig. 20 are gland fundaments in several stages of development. Some extend laterally for about

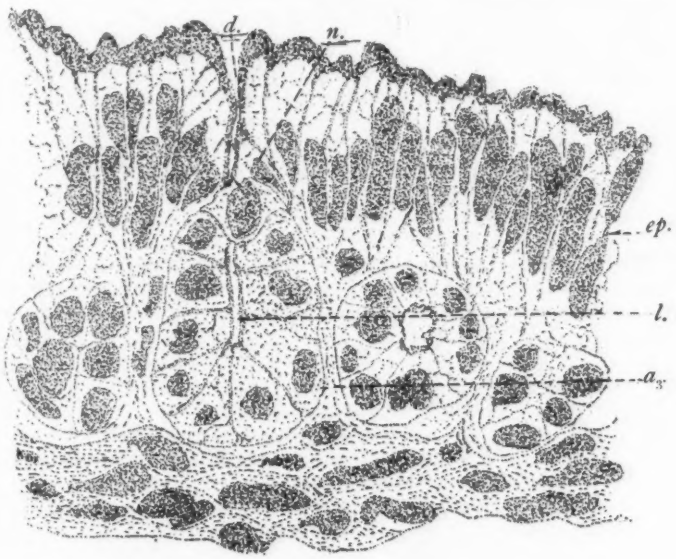


FIG. 14. a_3, gland showing lumen, *l.*; *d.*, deep indentation with a rearrangement of cells of the epithelium to form the duct.

30 micra. Here, as in *Desmognathus*, the epithelium appears to be made up of several layers of cells, the deeply staining nuclei (Figs. 19 and 20) being arranged at different levels. The cells lining the cavity of the stomach seem to be fewer in number on account of their great distention with yolk, consequently do not present the appearance of alternate layers to such a marked degree as was shown for *Desmognathus*. This orderly arrange-

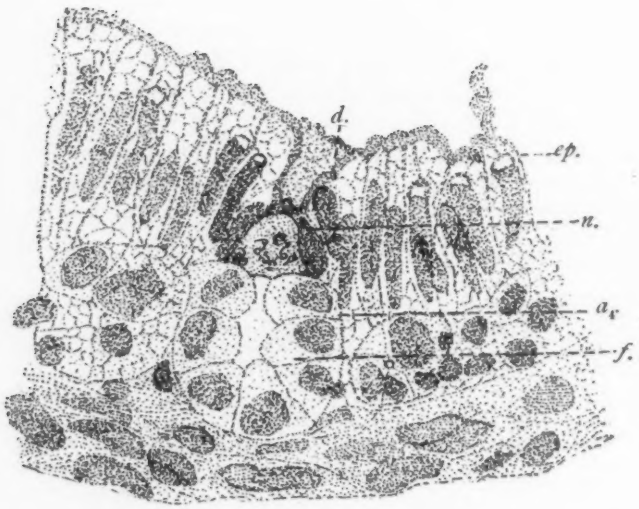


FIG. 15. a_4, gland showing the large clear cells, so characteristic of the earlier stages. The cells at the upper part are in process of division, showing the union of the duct, *d.*, with the fundus, *f.*, to form the neck *n*.

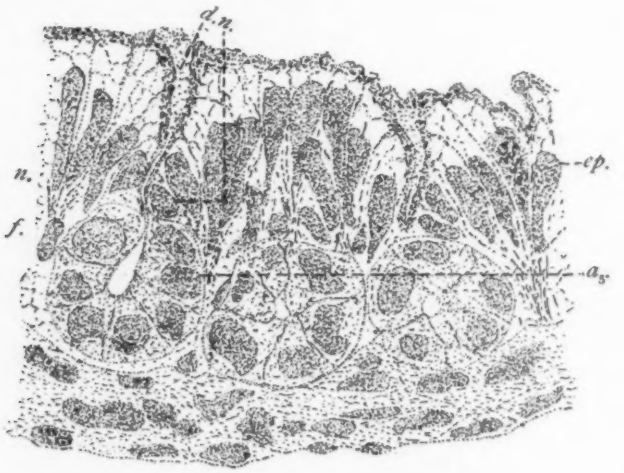


FIG. 16. a_5, section showing the opening of the gland to the surface.

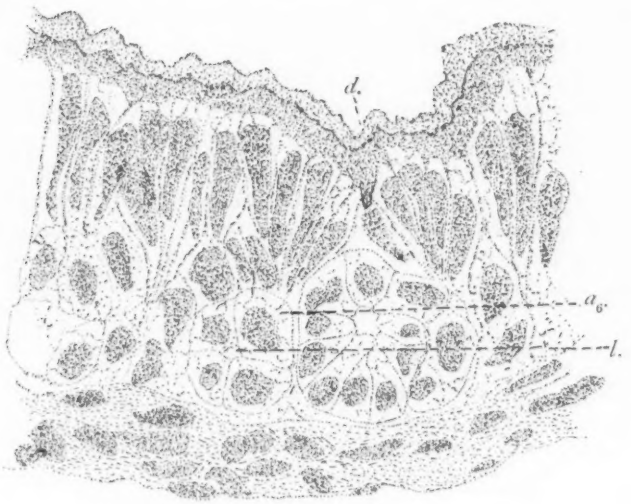


FIG. 17. a_6, same as Fig. 12, a_1.

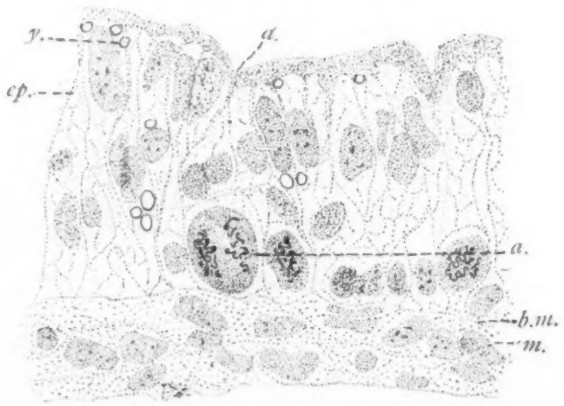


FIG. 18. Cross-section through the cardiac region of larval *Amblystoma* of 11 mm. total length. Gilson's, modified. *a.*, "ovoid cell," or gland anlage, in process of division; *b.m.*, basement membrane; *d.*, depression at the surface to form the future excretory duct; *ep.*, epithelium of the surface; *m.*, mesodermic tissue forming the muscular layers; *y.*, yolk spherules which obscure the cell outlines. Obj. 7, Oc. 2.

ment of nuclei at different levels also corresponds to the insinking at the surface, shown in all of the series of *Amblystoma* studied (Figs. 18, 19, 20, *d*). As the gland fundaments increase in size by cell division the yolk spherules become absorbed; the epithelium lining the stomach becomes narrower and lower, until in a larva of 12 mm. (Fig. 20) a stage comparable to Fig. 3 of *Desmognathus* is obtained. The yolk disappears from the glandular epithelium more rapidly than from the surface epithelium. Thus the origin of the fundus of the gland is more readily traced than the development of the excretory duct. Figs. 21 and 22 in

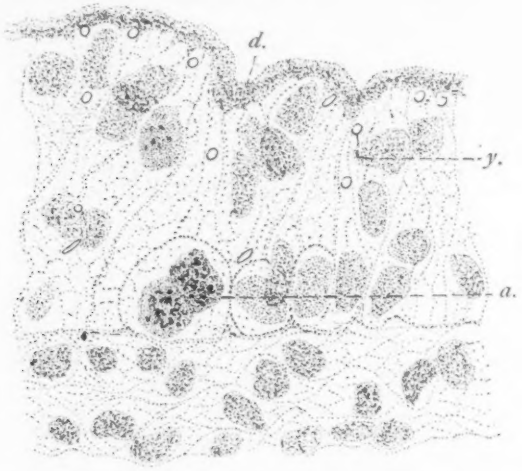


FIG. 19. Same as Fig. 18. The primary round cells now divided to form two. Obj. 7, Oc. 1.

a larva 16 mm. in length show slight insinkings or indentations at the surface. In the same figures the gland fundaments have progressed further in development than in the corresponding stage in *Desmognathus*. The fundus of the gland extends throughout several sections and the cells have already become arranged around a central lumen (Figs. 21 and 22, *l*). The cells which are to form the excretory duct and neck, although arranged around the gland as a central axis, do not present a marked opening. Instead of a narrow oval slit, as in a corresponding stage of *Desmognathus*, there is formed a shallow pit. From this

stage there takes place a rapid disappearance of yolk, a rapid multiplication of cells and a correspondingly rapid insinking of the surface epithelium to form the excretory duct. At the time of hatching there is, as before described for *Desmognathus*, a union of the gland fundus with the excretory duct, the cells showing marked mitoses at this point (Fig. 23, *n*).

There are several points in which the *Amblystoma* appears to differ from the *Desmognathus*. The cells of the surface epithelium

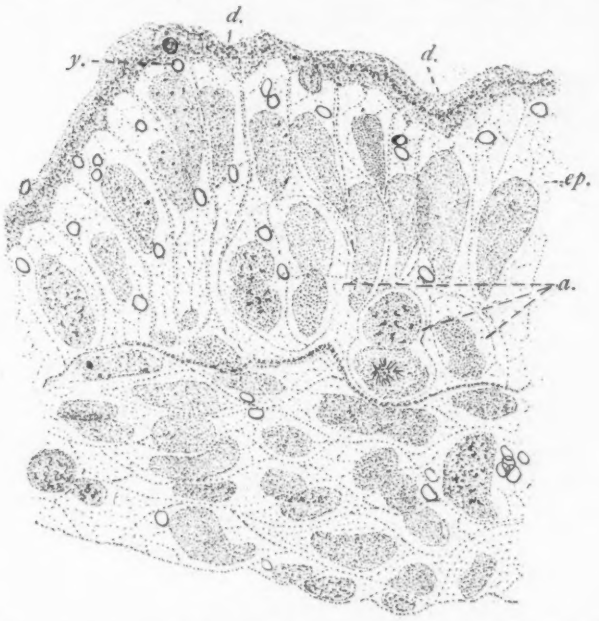


FIG. 20. Same as Fig. 18. Larva 13 mm. *a.*, groups of cells, two, three and four, by increasing in size, are pushing the epithelial cells aside. Thus the indentation, *d*, is being deepened. The yolk is also being absorbed, making the epithelium, *ep.*, narrower or lower. Obj. $\frac{1}{16}$, Oc. 2.

are fewer in number, being distended with yolk. The excretory duct is shorter, the glands opening on the surface of the stomach by very short necks. As the yolk is absorbed the columnar epithelium lining the stomach becomes lower; the gland fundaments increase more rapidly than the surface epithelium, as the

yolk disappears from them first, hence there is little space left for the formation of excretory ducts. The glands, then, are shorter and more flask-shaped than in the *Desmognathus*, though here too are found simple tubular glands.

Bensley ('00) describes the gastric glands of *Amblystoma* as "tubular downgrowths" of entoderm. "The anterior glands," he says, "are of a flask like shape and have a distinct lumen."

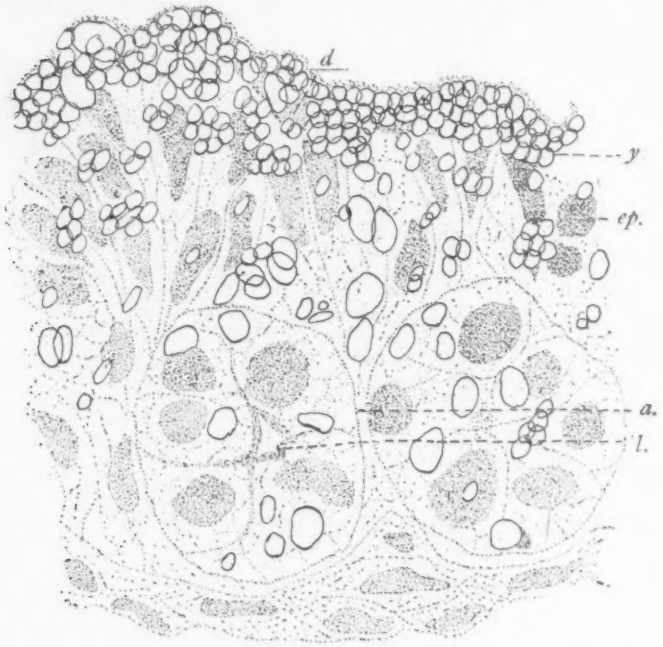


FIG. 21. *a.*, the gland fundament has increased in size very rapidly and now has a central lumen; *d.*, the duct has not developed to a corresponding extent, being as yet only a shallow pit; *ep.*, the epithelial cells are becoming radially arranged around the gland fundament as an axis; *l.*, central lumen; *y.*, the yolk is still present in the epithelial cells of the surface, though it has almost disappeared from the gland cells. Obj. $\frac{1}{12}$, Oc. 2.

"The posterior glands are simple tubes, composed of a simple layer of yolk-filled cells surrounding a cleft like lumen." From the glands described and figured by Bensley it seems probable that the "cleft like lumen," with the nuclei arranged as if sur-

rounding it, represents merely the excretory duct and, perhaps, the neck, while the fundus of the gland is to be sought for in the aggregation of cells he has portrayed at the base of his so-called gastric gland. Or this may be but one of the longitudinal ridges and depressions, cut transversely, which abound in the œsophagus but are less numerous in the stomach proper, though at an early stage they are seen here also.

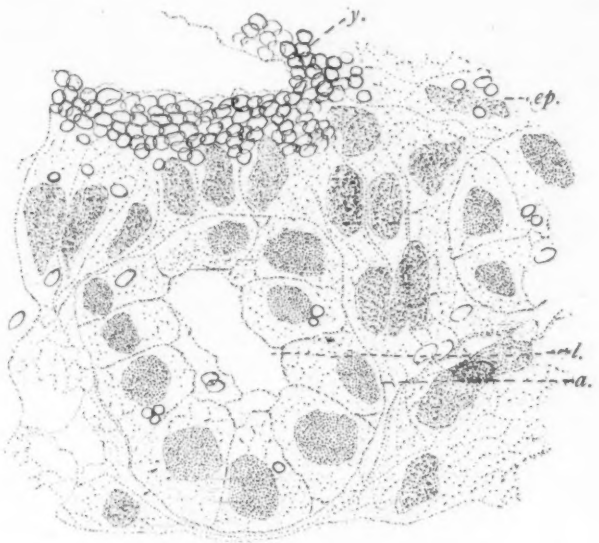


FIG. 22. *a.* shows a gland fundus almost fully formed while as yet the epithelial cells, *ep.*, are full of yolk, *y.*, and the duct, *d.*, is still a shallow pit; *l.*, lumen. The gland reaches almost to the free surface of the stomach. Obj. $\frac{1}{16}$, Oc. 2.

Pig.

The general course of development of the earlier stages of the gastric glands of the pig takes place in exactly the same manner as in the simpler forms already described. As early as in an embryo pig of 2 cm. total length, round, granular cells appear, differing from the remainder of the epithelial cells by their structural appearance and also by their characteristic staining reaction when stained with hematoxylin and eosin. The cells lining the stomach are so closely packed together that they appear to be

composed of several layers. Sections cut at 2 micra show that the epithelium is simple columnar; all the cells which reach the surface also rest upon the basement membrane, the "pyramidal cells" of Toldt ('80). In this respect the earlier stages in the pig also resemble those found in *Desmognathus* and *Amblystoma*

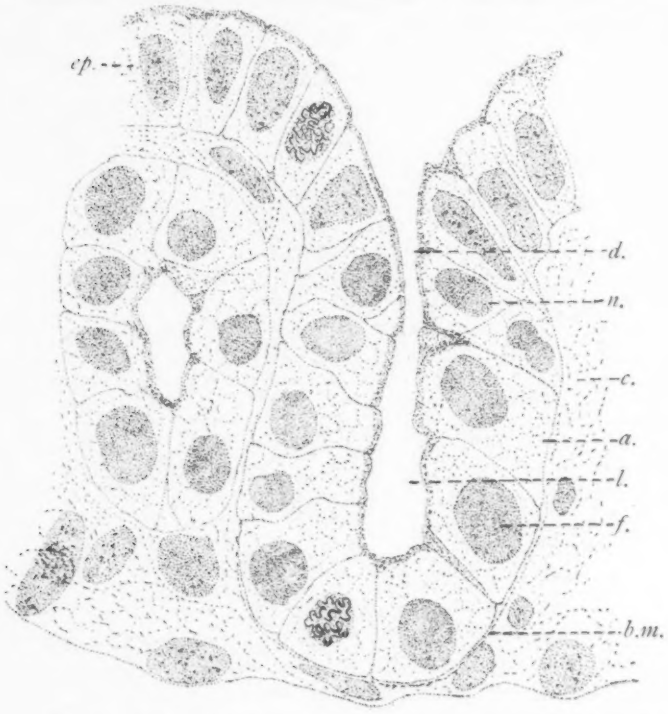


FIG. 23. Cross-section through the stomach of an *Amblystoma* of 16 mm. length. The yolk has all been absorbed. This shows the gland in the adult condition. Sections of larvæ of this size also show stages comparable to Figs. 13–18 of *Desmognathus*. Fixed in Flemming's fluid. *bm.*, basement membrane; *c.*, connective tissue; *d.*, duct, which is very short in *Amblystoma* due to its late development; *ep.*, epithelial cells; *f.*, fundus; *l.*, lumen; *m.*, muscular layer; *n.*, neck of gland. Obj. $\frac{1}{12}$, Oc. 2.

(Figs. 3, 19, 24). These round cells, appearing at the base of the epithelium, rest upon the basement membrane (Fig. 24, *a*). They then divide, as described before for *Desmognathus* and

Amblystoma, forming groups of two, three and four cells (Figs. 24, 25, *a*). While in both the other cases the epithelium was relatively thick, here it is very thin. For, as the gland anlages divide and increase in size, the epithelial cells gradually lose their stratified appearance, and become arranged in a single layer. The nuclei, instead of being arranged at different levels (Fig. 24, *a*), assume their place in the center of the cells (Figs. 28, 29, *ep*). Before this process is completed the gland fundaments cause a displacement of the surface cells, as in *Desmognathus* (Figs. 25, 26, *d*). The cells of the gland are arranged around a central

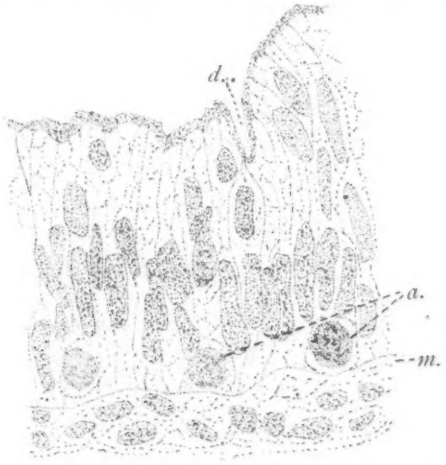


FIG. 24. Cross-section through the stomach of a pig of 62 mm. length. Fixed in mercuric chlorid. *a.*, "ovoid cells" or gland anlages; *d.*, insinking to form the duct; *m.*, connective tissue to form the submucosa. The muscular layers are not shown in any of the drawings of the pig. Obj. $\frac{1}{16}$, Oc. 2.

lumen, small to be sure, but still a lumen (Fig. 26, *l*). There is also a slight indentation at the surface, as the cells are forced out of their original position (Fig. 24, *d*). Thus in an embryo of 6.2 cm. length (Fig. 25, *d*) there is a stage similar to that shown in *Desmognathus* and *Amblystoma* (Figs. 3, 20, *d*). The insinking resembles that of *Amblystoma* rather than *Desmognathus*, as it is merely a shallow pit. This deepens as the gland fundaments increase in size and the connection between the fundus and the duct is then established (Fig. 27, *n*). Immediately after the

opening of these shallow pits or indentations into the lumen of the gland, and even in some cases before that has occurred, the cells of the surface epithelium and mesoderm begin to multiply

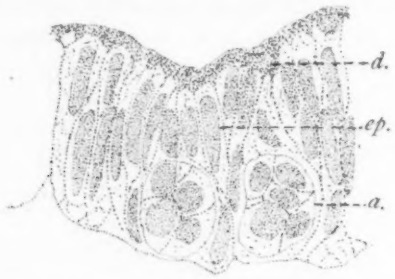


FIG. 25. Same as Fig. 24. *a.*, the original round cells have now divided, forming groups of several cells; *d.*, the indentations at the surface have deepened till they resemble those of *Amblystoma*; *ep.*, the epithelial cells are becoming radially arranged around the gland fundaments.

rapidly, projecting into the cavity of the stomach. These first outgrowths are, as Sewall ('79) describes them, in the form of short ridges, not of villi, as described by Brand ('78) and Negrini

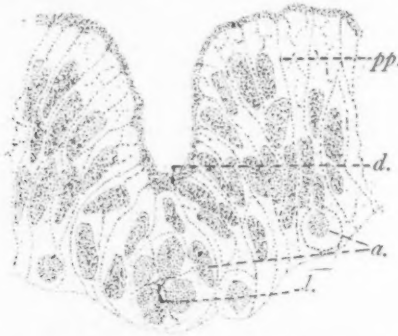


FIG. 26. Same as Fig. 24. Here the epithelium is increasing in size, pushed out also by increase in growth of the mesoderm to form the primary outgrowths or processes, *pp.* The glands are nearer the surface and the pits for the ducts, *d.*, are thus being deepened; *l.*, small central lumen.

('86). By continued outgrowths along these depressions and ridges the ridges intersect, resulting in a fine network. This is clearly seen with the naked eye in stomachs taken from pigs 14

cm. in length. These projections are termed "glandular processes" by Sewall because he considers them to be the first evidence of glandular formation, but it seems more probable that their primary object is to increase the epithelial surface of the

FIG. 27. Same as Fig. 24. *a.*, the gland fundament has here united with the duct, *d.*; *n.*, neck cells which are clearer and rounder than those of the surface epithelium; *ep.*, epithelial cells of the surface, not a part of the duct.

stomach. The glands, which are little more than pits or depressions, are seen at the tops of the ridges, at the sides of the ridges and in the depressions between the ridges. The glands are but little deeper than the original insinkings of the cells of the sur-

FIG. 28. Represents a section taken from the cardiac region. Similar sections are to be found in the fundus and also in the pyloric region; *f.*, fundus; *n.*, *d.*, neck and duct; *ep.*, epithelium. This may have been partially caused by artificial distension of the stomach. Formalin. Obj. $\frac{1}{18}$, Oc. 2.

face, but the fundus is readily distinguished as the cells are round, clear, staining intensely red with eosin, while the cells of the duct retain the appearance characteristic of surface epithelium

(Figs. 30, 31, 32, *f*, *d*). The duct cells are a little rounder and more granular than the rest of the stomach epithelium, hence they might more properly be termed neck cells (Figs. 27, 28, 32, *d*) as they correspond to the neck cells found in *Desmognathus*

FIG. 29 represents a section from the fundus of the same stomach as in Fig. 28. These two figures are taken from an earlier stage than Figs. 30 and 31, before the primary processes have begun to develop.

(Fig. 14, *n*). In this case the greater part of the future excretory duct still lies on the surface as surface epithelium. This is probably the true explanation, for the cells around the lumen of the glandular pits multiply and project out in all directions into the cavity of the stomach, forming secondary ridges. These secondary growths (Fig. 32, *sp*) appear on the sides and crests of the

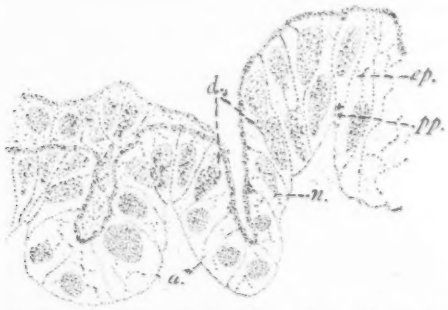


FIG. 30. Section through the pyloric region. Here is shown the same condition as in Fig. 32. Letters as in Fig. 28. Formalin. Obj. $\frac{1}{12}$, Oc. 2.

ridges already formed and in the depressions or hollows between them. The connective tissue also takes part in the formation of these secondary processes, projecting upwards as a central core, as shown in Fig. 32, *c*. At first these projections do little more than deepen the shallow pits, before described. As development proceeds further these processes become approximated (Fig. 32,

d) and, in this way, the excretory ducts are formed. The fundus of the gland, composed of deeply staining granular cells, remains near the basement membrane. In a later stage the fundus also elongates.

To recapitulate briefly: the fundus of the gland is formed from clear round cells lying at the base of the epithelium; the neck is formed from the epithelial cells which take part in uniting these to the surface epithelium; the excretory ducts are formed sec-

FIG. 31. Transection through the fundus of the stomach of a pig 16.5 cm. long. Same as in Fig. 32. Formalin. The mucosa is pushed out even straighter than in the preceding figure, so that the gland cells open almost on the surface of the stomach.

ondarily — by outgrowths of the single layer of epithelial cells, covering the processes of the primary outgrowths of the mesoderm, and also by a continued insinking of the surface epithelium.

It is not surprising that to these solid epithelial and mesodermic outgrowths, or projections, should have been ascribed the origin of the glands by Kölliker ('52), Laskowsky ('68), Brand ('78), Negrini ('86) and Salvioli ('90). For, in the pig at least, the round clear cells are very small, while these so-called "gland processes" are so marked and present so striking an appearance that it is little wonder that they should have attracted the most attention, and in them should have been sought the sole explanation of the glandular origin. In 1880 Toldt described, for the cat, solid epithelial masses "Ersatzzellen," as occurring at the base of the epithelium. These, he said, became arranged in a special form, degenerated in the center to form a vesicle or miniature gland. The vesicles then lengthened and divided to form

glandular pits. This is the nearest approach to a correct description anywhere found. Salvioli ('90) and Pilliet ('86) consider that the glands are formed by an insinking of the surface epithelium, as a cul-de-sac or vesicle at the base of the crypts or between the "gland processes." Sewall ('79) also described "ovoid cells" lying at the base of these crypts. He said that "the ovoid cells first appear in the deep part of the glands, and in older embryos they may be traced on, assuming more and more their completely developed characters, and becoming more numerous and extending farther up the gland." Instead of this

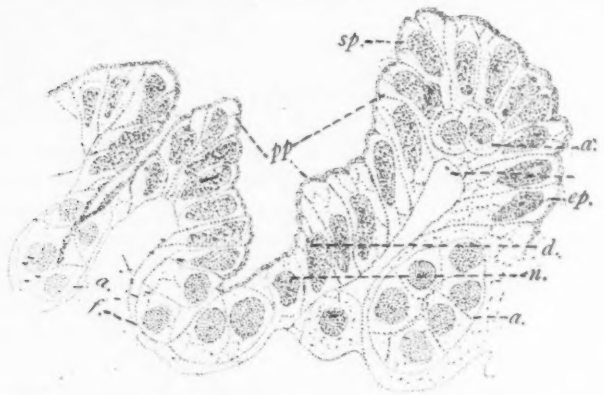


FIG. 32. Section through fundus of stomach of pig. Formalin. *a.*, gland anlage; *f.*, fundus of gland which has now, by the further growth of the "primary processes," *pp.*, been straightened out so that by looking down on a surface view, it is merely a shallow pit; *n.*, neck cells; *d.* later form the duct; *ep.*, epithelial cells of the surface; *sp.*, secondary process forming on the crest of a primary process. Obj. $\frac{1}{16}$, Oc. 2.

being the case, from one of the glands described by him, many, in reality, are formed, as was described above. It appears probable that the numerous "transitional forms" he finds between the "ovoid" and "embryonic" gland cells, are the neck cells, which occur at the place of union of the gland fundament and the excretory duct. The neck cells would at this stage be more marked and more numerous, relatively, than could be satisfactorily explained on other grounds. Thus, also, can be explained what appeared to him a "wholly unexpected phenom-

enon" — the presence of these ovoid cells throughout all the glands of the embryo stomach. These round cells do appear throughout the whole stomach wherever glands are to be formed, at the pyloric, cardiac and fundic regions in all three types examined. They are, as has been stated, the gland anlages, and are to form the functional portion of the glandular epithelium. A pig embryo of 10 cm. shows these round or "ovoid" cells at the base of the gland, resting upon the basement membrane (Fig. 32, *f*). A section through a pig of 14 cm. shows them still further depressed from the surface, appearing more truly gland shaped (Fig. 30, *f*). In this stage the glands are beginning to subdivide. Division takes place at the base of the gland and also along the sides of the fundus of the gland. Cells proliferate and project up into the lumen of the gland supported by connective tissue outgrowths. Several glands may thus come to open into one duct by the formation of these new outgrowths. These, in their turn, are subdivided by similar outgrowths. As all these open at the neck or in the fundus of the gland, compound glands are formed from what were originally simple tubes. This also takes place in the same manner in *Desmognathus* and *Amblystoma*.

In tracing the development of the earlier stages of the glands the whole stomach could be sectioned. In most cases the pancreas was left attached as it also assisted in rendering orientation easier. In pigs of more than 12 cm. total length portions were taken from different parts of the stomach, as cardiac, fundic and pyloric regions. Here, as in the earlier stages, glands were traced from section to section, every precaution being taken not to confuse them with one another, by taking accurate measurements of all the sections.

Summary.

The surface epithelium presents, at first, a stratified appearance, but soon becomes a single layer with nuclei approximately in the center of the cells. The stomach glands are formed from round granular cells lying at the base of the surface epithelium. These round cells divide and multiply, forming around a central lumen, while at the same time causing an insinking of the epithelium of the surface to form the excretory duct, by lateral displacement of

the surface cells. These then become united — forming at their junction the neck of the gland. So far all three forms agree. But in the pig the epithelial cells multiply and project into the cavity of the stomach to form ridges, supported by mesodermic outgrowths. On these ridges and in the pits between them are found the glands just described. They are lengthened by a further outgrowth of the epithelial cells about their lumen, also supported by connective tissue cores, and a slight downgrowth of the gland proper into the connective tissue beneath. In this way the excretory ducts are formed secondarily in the pig. These round or "ovoid" cells are not characteristic of any one region, but are found over the entire surface of the stomach in all three cases, wherever glands are to be formed. They are specialized very early, as they may be seen in *Desmognathus* and *Amblystoma* before the whole stomach is yet fully formed.

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A PRELIMINARY NOTE ON THE ABSORPTION OF THE HYDRANTHS OF HYDROID POLYPS.

H. F. THACHER.

Loeb has found that when pieces of *Campanularia* are placed in dishes of sea water, the polyps in contact with the glass undergo a transformation and disappear completely into the stem. This process, he states, is "due to contact, and is accomplished by the liquefaction and subsequent withdrawal of the protoplasmic mass." In taking up this subject, at the suggestion of Professor Morgan, my wish was to see whether in this case a study of the histology would support Loeb's theory of the liquefaction of the protoplasm as a result of contact: and also whether the process in *Campanularia* resembles that in other hydroids in which absorption occurs, but is due merely to the change from natural to laboratory conditions. For the latter point, in addition to *Campanularia*, I examined *Eudendrium* and a few cases of *Pennaria*, both of which forms also readily absorb their hydranths.

To see if by chance *Campanularia* would also absorb its hydranths when not in contact, I made a set of experiments, placing the splinters of wood on which the hydroids were growing in dishes, so that, as far as possible, the animals would be in a normal position. Under these conditions I found that the polyps were absorbed as rapidly as when touching the glass. These results show at least that contact is not essential to the production of this phenomenon, and suggest the likelihood that the absorption is due to the same cause in all cases.

The beginning of the degenerative changes are first shown by the appearance of large numbers of spherical granules in the digestive current, and by an increase in its rapidity. Shortly after, the polyp, which is to be absorbed, contracts into its cup, and the tentacles fold closely over it. Gradually the polyp becomes shorter and shorter, and the tentacles pass from the length found during ordinary contraction to a knob-like stage, and later are completely absorbed. Towards the close of this period the hypostome also disappears. At this time the digestive current

which has been forced periodically from one end to the other of the hydroid-colony, may, by distending the remains of the polyp, delay absorption for a number of hours. If, however, the pressure of the current is not great, the polyp grows gradually smaller until only a small ball of material is left in the cup, and this is then drawn down into the stalk. The whole process may occur in six hours, or may be prolonged for two days or more.

A study of the prepared material shows that changes begin first in the endoderm cells of the body of the polyp, into the cavity of which are thrown fragments of degenerating endoderm and gland cells. This continues for some time, and is accompanied by the contraction of the supporting lamella, as a result of which the ectoderm changes from a flattened to a columnar form. The cells of the hypostome round up rapidly at a comparatively late stage, and are set free into the digestive cavity; the lamella contracting as before. In the tentacles the endoderm is also in process of degeneration, and later, when a break comes in the lamella at the base of the tentacle, the cells pass into the body cavity. The ectoderm cells in this region are thrown into folds which, seen from the surface, might easily give the effect of being fused, as noted by Loeb; but I have not seen any signs of real fusion — only many cases where the cells of different tentacles are brought into close contact. During this time the lamella of the tentacle breaks, and masses of nettle cells and ectoderm pass through the break into the digestive cavity. The broken ends of the lamella now draw together and form a hollow shell, which is frequently much distended by the pressure of the digestive current on the elastic lamella. Degeneration continues by the slow turning in of ectoderm and endoderm cells, until only a small fraction of the original polyp remains, and this is then drawn through the opening at the base of the cup. There are no signs, either external or internal, of any drawing back of protoplasm to form a part of the stalk previous to the final stage, but at this time the strands of protoplasm connecting the cœnosarc and perisarc at the end of the stalk are broken, and in sections the masses of nettle-forming cells, which usually lie in the ectoderm just below the polyp, can be seen to have moved farther down the stalk.

Examination of *Eudendrium* and of *Pennaria* show that the process is the same as that in *Campanularia*, except in those secondary points to which the structural differences of the hydroids would give rise. There being no cup, the tentacles remain separate during absorption, so that there can be no question of fusion taking place. The lower row of tentacles of *Pennaria* persists somewhat longer than do those on the hypostome, but both ultimately disappear, and in neither form is there a withdrawal into the stalk until the polyp has almost entirely degenerated.

The results show that in these three hydroids the method of absorption is the same. No trace of liquefaction of protoplasm, or of withdrawal of the polyp as a whole can be found. The absorption takes place by the degenerating cells of the endoderm and ectoderm being turned into the digestive tract of the colony.